

SEQUENCE LISTING



<110> Brookhaven Science Associates Shanklin, John Whittle, Edward J.

- <120> Mutant Fatty Acid Desaturase and Methods for Directed Mutagenesis
- <130> CIP of 10/017,145 filed December 14, 2001 which was a CIP of
   09/328,550 filed on June 9, 1999, which was a CIP of 09/233,856
   filed on January 19, 1999
- <140> 10/822,370
- <141> 2004-04-12
- <150> 09/328,550
- <151> 1999-06-09
- <150> 10/017,145
- <151> 2001-12-14
- <150> 09/233,856
- <151> 1999-01-19
- <160> 19
- <170> PatentIn version 3.2
- <210> 1
- <211> 363
- <212> PRT
- <213> Ricinus communis
- <220>
- <221> misc\_feature
- <223> ricinus communis delta 9 18:0 Acyl ACP Desaturase
- <400> 1
- Ala Ser Thr Leu Lys Ser Gly Ser Lys Glu Val Glu Asn Leu Lys Lys  $1 \hspace{1.5cm} 5 \hspace{1.5cm} 10 \hspace{1.5cm} 15$
- Pro Phe Met Pro Pro Arg Glu Val His Val Gln Val Thr His Ser Met 20 25 30
- Pro Pro Gln Lys Ile Glu Ile Phe Lys Ser Leu Asp Asn Trp Ala Glu 35 40 45
- Glu Asn Ile Leu Val His Leu Lys Pro Val Glu Lys Cys Trp Gln Pro 50 55 60

Gln Asp Phe Leu Pro Asp Pro Ala Ser Asp Gly Phe Asp Glu Gln Val 65 70 75 80

Arg Glu Leu Arg Glu Arg Ala Lys Glu Ile Pro Asp Asp Tyr Phe Val 85 90 95

Val Leu Val Gly Asp Met Ile Thr Glu Glu Ala Leu Pro Thr Tyr Gln
100 105 110

Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala Ser 115 120 125

Pro Thr Ser Trp Ala Ile Trp Thr Arg Ala Trp Thr Ala Glu Glu Asn 130 135 140

Arg His Gly Asp Leu Leu Asn Lys Tyr Leu Tyr Leu Ser Gly Arg Val 145 150 155 160

Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser Gly
165 170 175

Met Asp Pro Arg Thr Glu Asn Ser Pro Tyr Leu Gly Phe Ile Tyr Thr 180 185 190

Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala Arg 195 200 205

Gln Ala Lys Glu His Gly Asp Ile Lys Leu Ala Gln Ile Cys Gly Thr 210 215 220

Ile Ala Ala Asp Glu Lys Arg His Glu Thr Ala Tyr Thr Lys Ile Val 225 230 235 240

Glu Lys Leu Phe Glu Ile Asp Pro Asp Gly Thr Val Leu Ala Phe Ala 245 250 255

Asp Met Met Arg Lys Lys Ile Ser Met Pro Ala His Leu Met Tyr Asp 260 265 270

Gly Arg Asp Asp Asn Leu Phe Asp His Phe Ser Ala Val Ala Gln Arg 275 280 285

Leu Gly Val Tyr Thr Ala Lys Asp Tyr Ala Asp Ile Leu Glu Phe Leu 290 295 300

Val Gly Arg Trp Lys Val Asp Lys Leu Thr Gly Leu Ser Ala Glu Gly 305 310 315 320

Gln Lys Ala Gln Asp Tyr Val Cys Arg Leu Pro Pro Arg Ile Arg Arg 325 330 335

Leu Glu Glu Arg Ala Gln Gly Arg Ala Lys Glu Ala Pro Thr Met Pro 340 345 350

Phe Ser Trp Ile Phe Asp Arg Gln Val Lys Leu 355 360

<210> 2

<211> 1092

<212> DNA

<213> Ricinus communis

<220>

<221> misc\_feature

<223> residues 138 to 1239 of open reading frame

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| aagaaaa  | attt   | ctatgcctgc  | acacttgatg                            | tatgatggcc                  | gagatgataa  | tctttttgac                     | 840          |  |
|--|--|---|---------------------------------------|-----------------------------|-------------|--------------------------------|--------------|--|
| cactttt  | cag  | ctgttgcgca  | gcgtcttgga                            | gtctacacag                  | caaaggatta  | tgcagatata                     | 900          |  |
| ttggagt  | tct  | tggtgggcag  | atggaaggtg                            | gataaactaa                  | cgggcctttc  | agctgaggga                     | 960          |  |
| caaaagg  | gctc   | aggactatgt  | ttgtcggtta                            | cctccaagaa                  | ttagaaggct  | ggaagagaga                     | 1020         |  |
| gctcaag  | ggaa   | gggcaaagga  | agcacccacc                            | atgcctttca                  | gctggatttt  | cgataggcaa                     | 1080         |  |
| gtgaago  | ctgt   | ag  |                                       |                             |             |                                | 1092         |  |
| <210> <211> <212> <213>  | 3<br>34<br>DNA<br>Arti   | ficial  |                                       |                             |             |                                |              |  |
| <220><br><223>   | amplification primer   |   |                                       |                             |             |                                |              |  |
| <220><br><221><br><223>  | <pre>misc_feature PCR primer; sequence flanking unique XbaI site at the 5' end of the open reading frame</pre> |   |                                       |                             |             |                                |              |  |
|  |  |   |                                       |                             |             |                                |              |  |
| <400><br>gtgagcg   | 3<br>ggat  | aacaatttca  | cacagtctag                            | aaat                        |             |                                | 34           |  |
|  | ggat  4 72 DNA   | aacaatttca  | cacagtctag                            | aaat                        |             |                                | 34           |  |
| gtgagcg<br><210><br><211><br><212>   | ggat<br>4<br>72<br>DNA<br>Arti   |   |                                       | aaat                        |             |                                | 34           |  |
| <pre>gtgagcg &lt;210&gt; &lt;211&gt; &lt;212&gt; &lt;213&gt; &lt;220&gt;</pre>   | 4 72 DNA Arti ampl   | ficial<br>dification p<br>c_feature<br>((57)<br>primer is a | orimer                                | e oligonucle                |             | nich "n" indi<br>leotide posit | .cates       |  |
| <pre>gtgagcg &lt;210&gt; &lt;211&gt; &lt;212&gt; &lt;213&gt; &lt;223&gt; &lt;220&gt; &lt;223&gt; &lt;221&gt; &lt;222&gt; &lt;221&gt; &lt;222&gt; &lt;223&gt;</pre> | 4 72 DNA Arti ampl   | efeature(57) primer is a                                    | orimer<br>a degenerate<br>f either C, | e oligonucle<br>A, T or G a | at that nuc | leotide posit                  | cates<br>ion |  |
| <pre>gtgagcg &lt;210&gt; &lt;211&gt; &lt;212&gt; &lt;213&gt; &lt;223&gt; &lt;220&gt; &lt;221&gt; &lt;222&gt; &lt;221&gt; &lt;222&gt; &lt;223&gt; </pre>            | 4 72 DNA Arti ampl  misc (56) PCR the  | eficial  cfeature(57) primer is a presence of caagacgtcg    | orimer<br>a degenerate<br>f either C, | e oligonucle                | at that nuc | leotide posit                  | cates<br>ion |  |
| <pre>gtgagcg &lt;210&gt; &lt;211&gt; &lt;212&gt; &lt;213&gt; &lt;223&gt; &lt;220&gt; &lt;223&gt; &lt;221&gt; &lt;222&gt; &lt;221&gt; &lt;222&gt; &lt;223&gt;</pre> | 4 72 DNA Arti ampl  misc (56) PCR the  | eficial  cfeature(57) primer is a presence of caagacgtcg    | orimer<br>a degenerate<br>f either C, | e oligonucle<br>A, T or G a | at that nuc | leotide posit                  | cates<br>ion |  |

<213> Artificial

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<220>
<223> amplification primer
<220>
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<400> 5
                                                                      31
gaaacaggtg caagtccgac gtcttgggca a
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      6
      26
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      amplification primer
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      misc_feature
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      PCR primer
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                                                                      26
gttttctgtc cgcggatcca ttcctg
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ttgataagtg ggaagggctt cttccgtt
                                                                      28
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<223> PCR primer is degenerate olignucleotide in which "n" indicates
       the presence of either C, A T, or G at that nucleotide position
       and in which "k" indicates either T or G
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       the presence of either T or G.
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                                                                      60
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tgaaac

66

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      PCR primer
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tccattcctg aaccaatcaa atattg
                                                                       26
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      PCR primer in a degenerate oligonucleotide in which "n" indicates
       the presence of either C, A, T or G at that nucleotide position
       and in which "k" indicates the presence of either T or G at that
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<223>
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      (49)..(51)
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       the presence of either C, A, T or G at that nucleotide position
       and in which "k" indicates the presence of either T or G at that
      nucleotide position.
ttgattggtt caggaatgga tnnkcggnnk gaaaacagtc cataccttnn kttcatctat
                                                                       60
acatcattcc
                                                                       70
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ccaaaagaaa aaggtaagaa aacccgggat ggctctcaag ctcaatcctt tcctttctc
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      15
<211>
      31
<212> DNA
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      artificial
<220>
<223> amplification primer
<400> 15
                                                                     31
ttgctctctc cctgagttcc ctgacttgct c
<210> 16
<211> 31
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<220>
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                                                                     31
gagcaagtca gggaactcag ggagagagca a
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       17
<211>
       38
<212> DNA
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|---|--------|--|----|
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|   | <223>  | amplification primer                     |    |
|   | <400>  | 17                                       |    |
|   | tccttt | gace tteettggge tetetettee ageettet      | 38 |
|   |        |  |    |
|   | <210>  | 18                                       |    |
|   | <211>  | 37                                       |    |
|   | <212>  | DNA                                      |    |
|   | <213>  | artificial                               |    |
|   | <220>  |  |    |
|   | +      | amplification primer                     |    |
|   | <400>  | 18                                       |    |
|   |        | tgga agagagagcc caaggaaggg caaagga       | 37 |
|   |        |  |    |
|   | <210>  | 19                                       |    |
|   | <211>  | 42                                       |    |
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|   | <213>  | artificial                               |    |
|   | <220>  |  |    |
|   |        | amplification primer                     |    |
|   | <400>  | 19                                       |    |
|   |        | cgat atcgagetet acagetteae ttgeetateg aa | 42 |
| • | -9~~   |  |    |